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Leader peptide → ← Domain 1
Human MLLFVLTCLLAVFPAISTKSP IFGPEEVNSVNSVITCYYPPTSVNRHTRKYRQAGARGGCITLISSEGY 55
Bovine -SRLE-A- -I- -VV-M- -T- -R- -K- -D- -Q-R-T-
Rat -R-SLEAL-VT- -SGV-Q- -QD-S-I- -D- -N-Y-A- -N-
Mouse -R-YLF-L-VT- -SGV- -Q-S-I- -D- -S-M-T- -N-
Possum -A -F-A- -LL-VV-M- -KQ-TG- -G- -Q-F-SS- -G- -F-L-NL-QS-E-IV- -N-F
Rabbit -A-L- -S-ATAQ-SLLGPSS- -G-VL- -D- -T- -S- -F- -EES-R-V- -A-T-
*
→ ← Domain 2 *
Human VSSKAGRANLTNFPENGTEFVNNIAQLSQDDSGRYKCGLSRGLSFDVSLVSSQGPGLLNDTKVYTVDLGRVTITNC 134
Bovine -DD-V- -S- -D-SH-THK- -S- -N- -D-AQASHAR-
Rat L-KE-S- -S-I- -S- -I- -H-T-E-T-S- -TTN- -F- -V-EF- -H- -K-I- -E-
Mouse L-KE-S- -S-I- -G-N-I- -E-T- -T-S- -TSN- -V-E-PS- -H- -K-I- -N- -E-
Possum -ERFS- -K- -N-S-LIQ-S- -EKE-I-L- -TSN- -IT- -GE-N- -P-N-E-IVTEV-K-S-
Rabbit T-QE-S- -GK- -D- -DK-E- -TVD- -T-N- -S- -V-V-G- -D-G-NVL- -K-E- -P-DV- -KQYESY- -T-
*
Human PFKTENAKRKSLYKQIGLYPVLVIDSSGYV NPNYTGRIRLDIOGTGQLLFSVVINQLRLSDAGQYLCOAGDDSSNSN 211
Bovine -TRA-SE- -C-KTIQCFQ-V-T- -SNS-KD-AHIS-L-NT-V- -RVK- -M-V- -AKAD
Rat -EG-HSK- -C-RR-EACEV- -TE- -D-S-KD-AI-FMK- -SROI-Y-N-SH-I-P- -L-V- -EGPSAD
Mouse -R-VPSK- -C-KTNQSC- -TEK- -S-I- -AK-FMK- -DLTV-Y-N-SH-THN- -L-I- -EGPSAD
Possum -QEQT-T-D- -F-C-KD-ESCA- -QE- -G-D- -A-S-S-SSRV-V-T-S- -IKRQ-V-M-V-GV-E- -DTGTI
Rabbit -TYATR-LK- -F- -VEDGEL- -I- -SKEAKD-R-K- -T-Q- -S-TAKE-T-T-KH-Q-N- -V-V-S-S-PTAE
*
→ ← Domain 3 *
Human KKNADQLVQLKPELVEYDLRGVSTFHCALGPEVANVAKFLCROSSGECNVVNTLGKRAFAFEGRILLNPQDKDGSF 290
Bovine -I-I- -E- -G- -S- -D-S- -P- -QKNV-GA-N- -I- -K-QQ- -Q- -VSV-K- -N-V-
Rat -N- -E- -L-K- -S- -E-D- -R- -D- -KNK- -T- -II- -D- -D- -T-R-DN-R-
Mouse -V- -A- -L-K- -S- -E-D- -R- -E- -Y- -MNK- -T- -II- -D- -D- -IT-K-DN-R-
Possum Q-V- -KL-E- -L-AE-G- -LN- -ST-S- -P- -QMRAT-T- -L-I- -SK-FTNN-TH- -FSHTTET-
Rabbit EQ-V- -RL-T-G- -L-GN-G- -E- -OS-D- -AVAS- -VR-G- -N- -IDSQ-TID- -FT- -KAEN-H-
*
→ ← Domain 4 *
Human SVVITGLRKEDAGRYLCGAHSDGQLQEGSPQIAQWLFVNEESTIPRSPVTKGVAGSSVAVLCPPYNRKESKSIKYWCLW 369
Bovine -H-S- -V- -QE-E-P-D-W-V- -TA-A-S- -R-G- -T-S- -P-DAN-A- -H-
Rat -L- -H- -Q- -S-LP- -R-V- -N-RS- -T-G- -T-V- -P-S- -L- -H-
Mouse -L- -H- -Q- -S-LP- -W- -T- -NRSS- -T-G- -TA- -P-S- -L- -H-
Possum -IM-QV- -E-V-H- -QE- -QPS-EG- -R-L- -S-T-V-K- -L- -PS-G- -TIT- -H-DP-KNNTL- -K-
Rabbit -A- -T-N- -VQ-N- -SGD- -T-LR- -IDVS- -P-L- -FP-G- -TIR- -P-R-D-HLQLY-
*
Human EGAQNGRCPLLVDS EGWVKAQYEGRLSLLEEPGNGTFTVILNQLTSRDAGFYWCLTNGDTLWRTTVEIKIEG EPN 445
Bovine -E- -R-E- -R-LI-E- -A-A- -DQ-T- -V-D- -R-IS- -L-VVQ- -S-
Rat -ADE- -V-GT- -QAL-QEG- -A-F-Q- -S-AY- -TQ-S- -D- -SR- -I-LQVA-ATKK-D-
Mouse -DG- -H-A- -GT- -QAL-QEE- -A-FDQ- -Y- -TE- -SR- -I-LQVA-ATR-
Possum EGS SH-TK- -L-M-DES- -VA-WD- -E-I- -PQ- -Y- -S-EHRKSS- -K-E-ND- -Q-
Rabbit -S-S-TRH- -G-L- -LQKD-T- -A-F- -S-V- -AE-E- -VSDD-ESLT-S-KLQ-VD-EPS-T-
*
↓ Bridge to IgA
← Domain 5 *
Human LKV PGNVTAVLGETLKVPCHEFPCKFSSYKWKNNWTCGQALPSQDEGSKAFVNCDSRLVSLTLNLVTRADEGW 523
Bovine -K- -W- -P- -LS- -Y-F- -S-R- -S- -T-ND- -Q- -S- -Q- -QV- -N-OT- -KE-
Rat -E-T-Q-A- -I- -FTS- -Y- -Y-Q- -S-D- -HI- -H- -ARQSS- -S- -QS- -QI- -M- -P- -KKE-
Mouse -E-T-Q-A- -I- -FTS- -Y- -Y-Q- -S-K- -HI- -H- -ARQSS- -S- -QS- -Q- -M- -P- -SKE-
Possum -LIA-KT- -Q- -QS- -TIS- -Y- -S- -Q- -ET- -T- -E- -S- -Q- -D-NQ- -N- -S- -SDH-
Rabbit I OKF- -Q- -PVEIT- -YF-S- -D- -DH- -ED- -TKLSSSGD- -L-K-N- -NLVLT- -DS- -ED-
*
→ ← Domain 6 *
Human YWGVKQGHFYGETAAVY VAVEERKA AGSR DVSLAK ADAAP DEKVLDSGFEIENKAIQDPRL 586
Bovine -E-PR- -L- -S-V- -K-Q- -G-QV- -K- -AGAAIQ-RAG- -Q- -LL- -SF-
Rat -E-QV- -T-I- -TR- -PHINPTD-NAR-KD- -E-EAME-SV- -D- -NL-
Mouse -T-QT- -T-I- -I- -SHVNPDT-NAR-KV-L- -E-E-V-SIS-K- -PN-GP-
Possum -N-QN- -I-S- -S- -EV- -S- -NAIQPTN-VLN-ED-V- -E-KV-
Rabbit -A-D- -EFE-V- -RVELTEPAK- -PA-VPDPKAKA PAP-EEK-K-V- -SAQ-KAVVPVIVK-A- -VV-K-
*
↓ SC cleavage? *
Human FAEEKAVADTRDQAGDSRASVDSGSEEQ GGSSRALVSTLVPLQLVLAAGAVVFAARHRKNVORVSIRSYPDI 663
Bovine -K-SV- -K-A-G- -PG-PA-P-RPTGYS -K- -V- -LA-V- -I- -V- -I- -V- -I- -V-
Rat -D-REIQAGV-G-QEN- -GNA-AGG-S- -KV-F- -A- -T- -H- -V- -M- -S-
Mouse -N-REIQAGV-G-QEN- -G-A-ADG-SRSS- -KV-F- -I- -V- -M- -S-
Possum RKG-IE- -PT-L- -T-HS- -V- -A- -T- -L- -IX- -RWRF- -V- -G-
Rabbit L- -V- -QSAR- -P-S- -AS-ASG-S- -AKV-I- -A- -M- -AI- -R- -G-
*
Basolateral Targeting
Calmodulin Binding
*
Human SMSDFENS REFANDNMGASSITQETSLGGKEEFVATTESTTETKEPKKAKRS SKKEAEAYKDFLLQSSVAAEA QGPGQEA 746
Bovine -D-EGR- -PEA- -D-AT- -D-V-S- -D- -FTT- -AKNL-SA-T-N- -T-
Rat -G-R- -DL-G- -TPD- -V-E-D- -IET- -C-PE-S- -D- -SA- -W- -I- -QV-H-
Mouse -A-K- -DL-G- -TPD- -Q-VIE- -D-I-T- -C-A-PE-S- -D- -SA- -I- -QV-H-
Possum -EL-NP-Q- -E-D- -V- -T- -ED- -IITQ- -NPE-IQQ- -QV- -D- -TA- -NNM-N-PLD- -SN-
Rabbit -L- -I- -PS-CPDAR- -A- -D-LATA- -V-IE- -D- -DL- -SA- -N- -I- -H- -K-
*
↓ Avoid Degradation →
Rapid Endocytosis

Figure 1

10	20	30	40	50	60
MLLFVLTCLL	AVFPAISTKS	PIFGPEEVNS	VEGNSVSITC	YYPTSVNRH	TRKYWCRQGA
70	80	90	100	110	120
RGGCITLISS	EGYVSSKYAG	RANLTNFPEN	GTFVVNIAQL	SQDDSGRYKC	GLGINSRGLS
130	140	150	160	170	180
FDVSLEVSQG	PGLLNDTKVY	TVDLGRTVTI	NCPFKTENAQ	KRKSLYKQIG	LYPVLVIDSS
190	200	210	220	230	240
GYVNPNYTGR	IRLDIQGTGQ	LLFSVWINQL	RLSDAGQYLC	QAGDDSNSNK	KQADLQVLKP
250	260	270	280	290	300
EPELVYEDLR	GSVTFHCALG	PEVANVAKFL	CRQSSGENCD	VVNTLKGRA	PAFEGRILLN
310	320	330	340	350	360
PQDKDGSFSV	VITGLRKEDA	GRYLCGAKSD	GQLQEGSPIQ	AWQLFVNEES	TIPRSPTVVK
370	380	390	400	410	420
GVAGSSVAVL	CPYNRKESKS	IKYWCLWEGA	QNGRCPLLVD	SEGWVKAQYE	GRLSLLEEPG
430	440	450	460	470	480
NGTFTVILNQ	LTSRDAGFYW	CLTNGDTLWR	TTVEIKIIEG	EPNLKVPGNV	TAVLGETLKV
490	500	510	520	530	540
PCHFPCKFSS	YEKYWCKWNN	TGCQALPSQD	EGPSKAFVNC	DENSRLVSLT	LNLVTRADEG
550	560	570	580	590	600
WYWCGVKQGH	FYGETAAVYV	AVEERKAAGS	RDVSLAKADA	APDEKVLDSG	FREIENKAIQ
610	620	630	640	650	660
DPRLFASEKA	VADTRDQADG	SPASVDSGSS	EEQGGSSPAL	VSTLVPLGLV	LAVGAVAVGV
670	680	690	700	710	720
ARARHRQVND	RVSIRSYRTD	ISMSDFENSR	EFGANDNMGA	SSITQETSLG	GKEEFVATTE
730	740	750	760		
STTETKEPKK	AKRSSKEEAE	MAYKDFLLQS	STVAASEAQDG	PQEA	

Figure 2

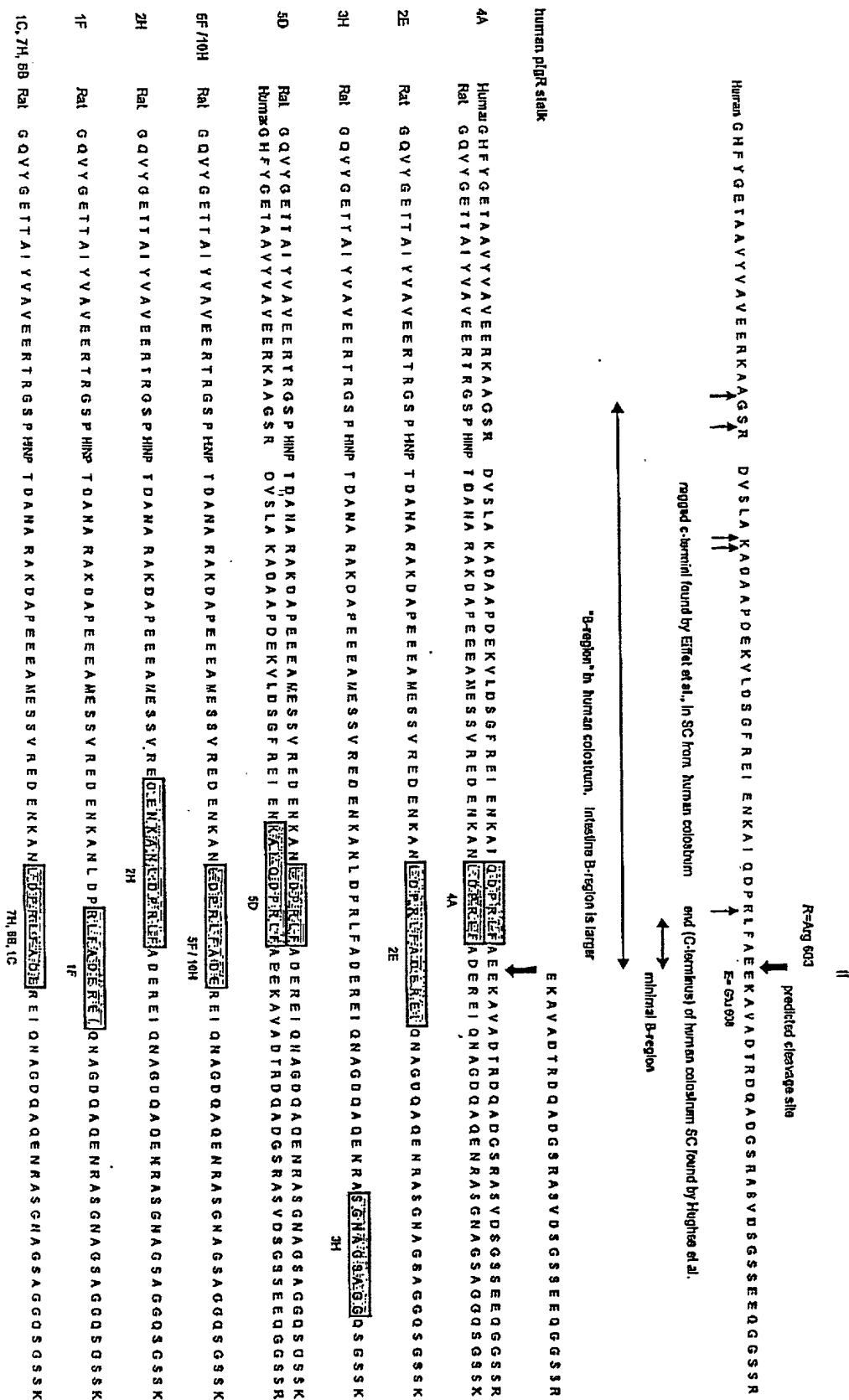


Figure 3

Pelb/4AF/myc/6HIS

PelB leader		FLAG	Heavy chain FR 1		CDR 1
MKYLLPTAAAGLLLLAAQ		PAMADYKAKQ	VLVQSGGGLVQPGGSLRL	SCAASGFTTSS	YAMS
FR 2	CDR 2		FR 3		
WVRQAPGKGLEWVS		AISSGGSTYYADSVKGRFTISRDN	SKNTLLYLQ	MNSLR	AEDTAVYYCAR
CDR 3	FR 4	linker		Light chain FR 1	
SFTVNSGYFQHWGQ		FTLVTVSSGGGGSGGGSGGGSEIVLTQSPSTLSASIGDRVTTTC	RASEGIYHWL		
FR 2	CDR 2	FR 3		CDR 3	
WYQQKPGKAPKLLITYKASSIASGVP		SRFSGSGGTDFTLTITSSIQPEDFATYYQ	HYDSTPPT		
FR 4	myc	6 HIS			
FGQGTKVDIKRAAAEQKLISEEDIN		GAHHHHHH			

The amino acid sequence of the secreted form of the ScFv 4AF is shown. The ScFv consists of a pelb leader (for secretion in *E. coli*), a FLAG epitope tag, a heavy chain variable region, a linker sequence (GGGS repeated three times), a light chain variable region, a myc epitope tag and a 6HIS tag (for purification by Immobilized Metal-ion Affinity Chromatography (IMAC)). The framework (FR) and complementarily-determining regions (CDR) of the heavy chain and light chain are indicated.

Figure 5